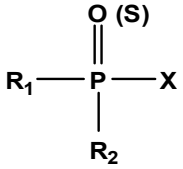
SYNOPSIS

(1) TITLE OF THE SYNOPSIS

MECHANISTIC STUDY OF EFFICIENCY AND REACTIVITY OF NOVEL OXIME REACTIVATOR OF CHOLINESTERASE INHIBITED BY ORGANOPHOSPHORUS TOXICANTS.

(2) INTRODUCTION

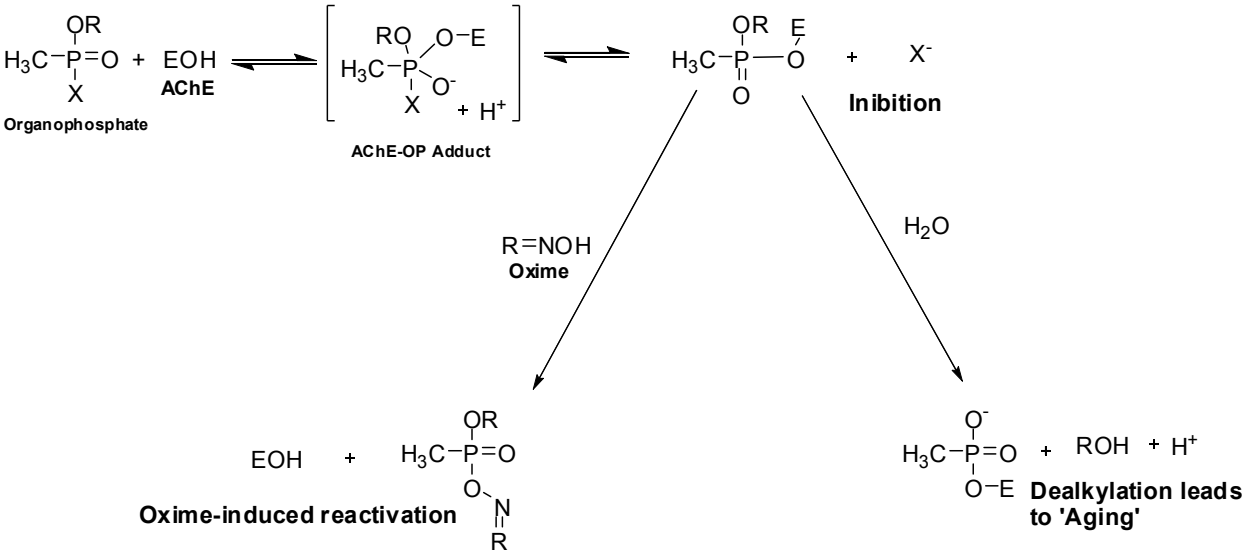
Organophosphorus (OP) based chemical warfare agents (CWA) and pesticides (Fig. 1) are rigorous public health issue with huge fatalities and mortality rates annually all over the world [1]. Inspite of extensive efforts to limit or ban the misuse of OPCs by several countries, their threat continuous as evident from the terrorist attack using sarin at Aum Shinrikyo, Tokyo (1995) Iran-Iraq war and Syria attack [2]. The main target of these uncharged and lipophilic OP is enzyme acetylcholinesterase (AChE). The primary function of AChE is the termination of nerve impulse transmission at cholinergic synapses by rapid hydrolysis of the neurotransmitter acetylcholine (ACh) [3]. AChE is found in several types of tissues such as cholinergic and non-cholinergic fiber nerves and muscles, central and peripheral nervous system tissues [4]. OP lead to the inhibition of physiological function of enzyme acetylcholinesterase (AChE) via the formation of a covalent bond at the serine hydroxyl group located at the deep narrow gorge of the AChE [5] (Scheme 1). The inhibited AChE is unable to hydrolyze the neurotransmitter ACh and accumulates in the synaptic cleft resulting in cholinergic over stimulation, respiratory disorders, paralysis and sometimes death [6]. Therefore it is essential to design most efficacious oximes for the development of antidotes for intoxications by organophosphates.



Sarin: R_1= Methyl; R_2= O-isopropyl; X= F
Soman: R_1= Methyl; R_2= O-pinacolyl; X= F
VX: R_1= Methyl; R_2= O-ethyl; X= F
DFP: R_1= Methyl; R_2= O-isopropyl; X= F
Paraoxon: R_1= O-ethyl; R_2= O-ethyl; X= p-nitrophenyl
Malaoxon: R_1= O-methyl; R_2= O-methyl; X= S-(1,2diethoxycarbonyl)ethyl

Fig. 1 Chemical structures of selected organophosphates

Customary treatment of OP poisoning underlines the use of an antimuscarinic agent e.g. atropine inclusive of oxime based reactivators [7]. Oximes as α- nucleophiles are important tool for the post treatment of OP intoxications as they reactivate phosphylated cholinesterase by cleaving the phosphoester bond between the active serine residue and OPs [8] (Scheme 1).



Scheme 1. Schematic representation of inhibition of AChE by an organophosphate and subsequent post-inhibitory reaction with its reactivation.

Several potential oxime reactivators have been designed and synthesized from past decades. At present, only few oximes e.g. 2-PAM and Obidoxime are used clinically and considered to be little ineffective against certain CWA and pesticides. This inadequacy of conventional oximes encouraged the synthesis of novel oximes with several structural modifications. Several research groups synthesized thousands of oximes in search of a better cholinesterase reactivator against all kinds of OP [9]. In this regard several studies has undertaken as physicochemical properties (acid dissociation constant; pK_a, lipophilicity; log P) and reactivation kinetics (k_r, K_D and k_{r2}) [10].

As oxime takes part in the form of oximate anion (deprotonated group) hence pK_a values should be considered while searching for new oxime reactivators. Lipophilicity (log P) is a useful parameter for the prediction of drug transport properties and is also important for evaluating the ability of different drugs to penetrate through the BBB and lipid bi-layer membrane [11]. Overall efficiency of reactivator is quantified second order rate constant (k_{r2}: ratio of reactivity rate constant (k_r) and K_D: apparent dissociation constant). K_D predicts the affinity of oximes towards the phosphylated AChE whereas k_r denotes the detachment of OP-residue from AChE-OP-oxime complex (as

demonstrated in eqs 1 and 2). Understanding the mechanism of reactivation will help to design some better nerve agent antidotes.

$$[EP] + [OX] \underset{}{\overset{K_D}{\rightleftharpoons}} [EPOX] \xrightarrow{k_r} [E] + [POX] \text{.....(1)}$$

$$[E] + [POX] \xrightarrow{k_{pox}} [EP] \text{ .....(2)}$$

In our proposed work physicochemical parameters and nucleophilicity of different novel oximes (Fig. 2) will be studied and their efficacy will be compared with conventional oximes. Reactivation kinetics of investigated reactivators will be performed against CWA and pesticide inhibited-AChE to get better insight into the structural requirements for recognizing a superior reactivator. Further, an attempt will be made to study reactivation kinetics with the help of binary mixtures of different oximes in their appropriate ratios. Combination of oximes may act as a promising tool in search of competent reactivators against OP-intoxication.

(3) A BRIEF REVIEW OF THE WORK ALREADY DONE IN THE FIELD

Several potential oxime reactivators have been designed and synthesized in the past decades [12-15]. The search of a superior oxime based reactivator dates back to the early 1950s with the discovery of 2-PAM [16]. Later on many bis-pyridinium derivatives like trimedoxime (TMB-4), obidoxime, HI-6 were synthesized [17]. All above mentioned oximes are standard oximes which are used in defence for reactivation of OP-inactivated AChE [18]. Kuca et. al [19] and Musilek et. al [20] synthesized large varieties of mono- and bis-pyridinium oximes (K-Oximes). In addition to these Worek, Acharya, and Taylor made special contributions [21-23]. Acharya and coworkers synthesized a series of carbmoyl bis-pyridinium mono-oximes with xylene linker and studied reactivation potential against OP-inhibited AChE [24]. Recently, Musilek and group synthesized butene linked bis-pyridinium oximes with different functionalities [25]. Worek and research group tested the ability of combinations of commercially available oximes (HI-6 + obidoxime) to reactivate CWA-poisoned huAChE [26] and reported the strategy as a promising approach. *In-vitro* reactivation of paraoxon inhibited electric-eel and huAChE with bis-quaternary pyridinium oximes have been evaluated by Thompson et. al [27]. Design, synthesis and evaluation of α-nucleophiles for the hydrolysis of nerve agents and their application to the reactivation of phosphorylated acetylcholinesterase have been documented by Renard et. al [28].

Ghosh and coworkers studied *in-vitro* reactivation kinetics of xylene linked mono-oximes against pesticides (paraoxon and DFP) and nerve gases (VX and Sarin) inhibited-AChE [29]. Inspite of these tremendous efforts, till date no single oxime is available which can be effective against all kinds of OP-poisoning, hence researches are ongoing to develop a universal reactivator [30]. This fact concludes that there is an urgent need to develop an effective medical treatment for the whole range of OP and nerve agents [31].

(4). OBJECTIVES

The main objectives of the proposed research work are as follows:

I. To determine acid dissociation constant (pK_a) and lipophilicity (log P) of some mono- and bis-pyridinium oximes.
II. Study of *in-vitro* reactivation kinetics of some novel oxime reactivators against organophosphates- inhibited AChE.
III. Study of reactivation of OP-inhibited AChE with different combinations of oximes.
IV. Surfactant mediated hydrolysis of carboxlyate, phosphate and sulphonate esters by oximate and hydroxamate anions.

5. NOTEWORTHY CONTRIBUTION IN THE FIELD OF PROPOSED WORK

Organophosphorus (OP) compounds have been widely used as pesticides and developed as chemical nerve agents such as soman, sarin, tabun, VX, and others [32]. Exposure to even small amounts of OP compounds can be fatal. The accumulation of these toxic compounds in the environment is a recognized ecological threat existing in the global scenario and resulting in serious health problems. All over the world many scientists and their groups are synthesizing novel oximes for reactivators of inhibited AChE. The efficiency of the oximes is depending on the nucleophilicity and physiological parameters [33]. In order to accomplish the structure activity relationships (SAR) of oximes to reactivate OP inhibited AChE, it is important to study the physicochemical properties and reactivation rate constants of variety of AChE reactivators [34]. In the last few years several noteworthy contributions have been made by our research group to study the structure, kinetics and properties of various oximate ions and their role in detoxification chemistry.

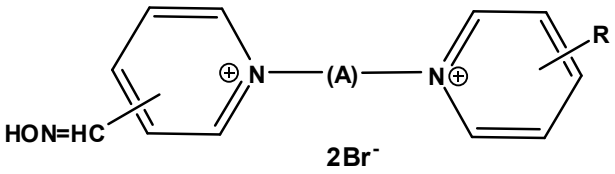
6. PROPOSED METHODOLOGY:

The entire work will be carried out in the following steps:

I. Determination of acid dissociation constant (pK_a) and lipophilicity (log *P*) of some mono- and bis-pyridinium oximes.

The acid dissociation constants (pK_a) and partition coefficient (log *P*) of tested cholinesterase reactivators will be determined using UV-VIS spectrophotometer by following Albert and Sergeant and Shake flask methods respectively.

II. Study of *in-vitro* reactivation kinetics of some novel oxime reactivators against organophosphates- inhibited AChE.



Where,
R= $CONH_2$, COOH, $COCH_3$, COOEt, OH, CH_2OH etc.
A= alkanyl, alkeneyl, alkyneyl, xylenyl etc.

Fig. 2 General Structure of some novel oximes

The neucleophilic potency of oxime reactivators against OP inhibited-AChE will be studied using modified Ellman's method. Organophosphates like paraoxon, methyl paraoxon and other chemical warfare agents will be used as AChE inhibitors. Human AChE and electric eel AChE will be used as enzyme source.

III. Reactivation efficiency of combination of oximes reactivators against OP-inhibited AChE.

The new strategy has been designed by applying binary mixtures of oximes in different ratios for the reactivation of inhibited AChE. The observed results of reactivation capability will be compared with ability of single reactivator in terms of reactivation parameters.

IV. Surfactant mediated hydrolysis of carboxlyate, phosphate and sulphonate esters by oximate and hydroxymate anions.

Micellar catalyzed kinetic studies of hydrolysis of different simulants of OP (phosphate, thiophosphate, carboxylate and sulphonate esters) will be studied with the help of different oxime nucleophiles (oxime based functionalized surfactants) and hydroxamate α-nucleophiles.

(7). EXPECTED OUTCOME OF THE PROPOSED WORK

There are large varieties of reactivators synthesized but no single oxime is available as universal antidote against several structurally different organophosphates (nerve agents and pesticides) intoxications. Hence the proposed strategy as combinations of either oximes may provide better reactivations results. The findings may be of vital importance to the defence and academic research. The present study will enrich our understandings to design some promising reactivators as antidotes.

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9. LIST OF PAPERS COMMUNICATED / SUBMITTED / PUBLISHED / IN JOURNALS / CONFERENCE / SYMPOSIUM

1. Regeneration of Pesticides Inhibited Cholinesterase
   Arvind Kumar Sahu, Rahul Sharma, Bhanushree Gupta and Kallol K Ghosh**,** National Conference on Recent Trends in Chemical Sciences 23-25 Jan. **2014**, SoS in chemistry, Pt. Ravishankar Shukla University, Raipur. (Presented Poster)

2. The influence of combination of oximes on the reactivating and therapeutic efficacy of antidotal treatment of organophosphate poisioning.
   Rahul Sharma, **Arvind Kumar Sahu,** Bhanushree Gupta and Kallol K Ghosh, 16 th Chemical Research Society of India, IIT Powai, Mumbai, 6-9 Feb. **2014**.

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Signature of Supervisor

(Dr. Kallol K. Ghosh)

Dr. Kallol K. Ghosh
Professor and Head
School of Studies in Chemistry
Pt. Ravishankar Shukla University
RAIPUR (C.G.)

Signature of Candidate

(Mr. Arvind Kumar Sahu)

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